Amendments to the Specifications:

Please replace the paragraph starting at page 2, line 22 of the specification with the following amended paragraph:

Alternatively, *M. ptb* can be confirmed through PCR to identify the presence of the species-specific DNA fragments. To date, only three subspecies-specific DNA fragments have been identified in the *M. ~~a.~~ ptb* genome.

Please replace the paragraph starting at page 5, line 20 of the specification with the following amended paragraph:

Preferably, the host is *E. coli*~~E. coli~~.

Please replace the paragraph starting at page 10, line 26 of the specification with the following amended paragraph:

Accordingly, in another aspect the present invention provides a method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting a sample with a polypeptide of the invention or a composition comprising a polypeptide of the invention and detecting a response indicative of the presence of *Mycobacterium avium ~~atrium~~ subspecies paratuberculosis*.

Please replace the paragraph starting at page 23, line 28 of the specification with the following amended paragraph:

Polypeptide sequences may be aligned, and percentage of identical amino acids in a specified region may be determined against another sequence, using computer algorithms that are publicly available. The similarity of polypeptide sequences may be examined using the BLASTP algorithm BLASTP software is available on the NCBI anonymous FTP server ~~(ftp://ncbi.nlm.nih.gov) under /blastlexecutables/~~. The use of the

BLAST family of algorithms, including BLASTP, is described at NCBI's website ~~at URL http://www.nobi.nlm.nih.gov/BLAST/newblast.html~~ and in the publication of Altschul, Stephen F., et al. (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res. 25:3389-34023.*

Please replace the paragraph starting at page 26, line 9 of the specification with the following amended paragraph:

Variants or homologues of the above polynucleotide sequences also form part of the present invention. Polynucleotide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another sequence, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. The BLASTN software is available on the NCBI anonymous FTP server ~~(ftp://ncbi.nlm.nih.gov) under /blastlexecutables/~~. The BLASTN algorithm version 2.0.4 [Feb. 24, 1998], set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, is described at NCBI's website ~~at URL http://www.ncbi.nbm.nih.gov/BLAST/newblast.html~~ and in the publication of Altschul, Stephen F, et al (1997). "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402. The computer algorithm FASTA is available on the Internet at the ftp site ~~ftp://ftp.virginia.edupub/fasta/~~. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention The use of the FASTA algorithm is described in the W R Pearson and D. J. Lipman, "Improved Tools for Biological Sequence Analysis," Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988) and W. R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA," Methods in Enzymology 183:63-98 (1990).

Please replace the paragraph starting at page 31, line 1 of the specification with the following amended paragraph:

In addition to the vaccine embodiments described above, it will be understood that a live vaccine could also be employed to protect a host against M. ptb infection or Johne's disease. In such a live vaccine, the DNA molecule of the invention encoding the protein, functionally equivalent variant or active fragment thereof, is incorporated into the genome of an attenuated carrier organism. A number of carrier organisms are known in the art which are suitable for this purpose with examples being *Salmonella and Vaccinia* ~~Salmonella and Vaccinia~~ virus.

Please replace the paragraph starting at page 36, line 2 of the specification with the following amended paragraph:

Approximately 3 mg of lyophilized ~~lyophilised~~ M. ptb (New Zealand field isolate ATCC 53950) was resuspended in 0.6 ml of extraction buffer (100 mM NaCl, 25 mM EDTA (pH 8.0), 10 mM Tris.Cl pH 8.0, 0.5%(w/v) SDS) before adding 200 µg of proteinase K (Roche Molecular Biochemicals, Germany). The mixture was incubated at 50°C for 18 h and then 100 µl of 5 M NaCl and 120 µl of 6.7%(w/v) cetyltriniethylammornium bromide (Aldrich Chemical Company, USA) in 0.5%(w/v) NaCl was added. The digest was mixed with an equal volume of 25:24:1 phenol:chlorofomi:isoamyl alcohol and centrifiged at 15,800 x g for 5 min. The aqueous phase was collected and the phenol:chloroform:isoamyl alcohol extraction was repeated, as above. The aqueous phase was mixed with an equal volume of chloroform and centrifuged again. The DNA was precipitated with the addition of 1 volume of 100% isopropanol to the collected aqueous phase. After 18 h at -20°C., the DNA was pelleted by centrifugation at 15,800 x g for 30 min at 4°C. The pellet was washed with 1 ml of 70% ethanol, air dried at room temperature and resuspended in 100 µl of TE buffer containing 100 µg/ml RNAse A (Life Technologies Inc., USA) and incubated for 18 h at 37°C. The DNA concentration was calculated based on absorbance at 260 nm. To check

the condition of the DNA, an 8 µl sample (~11 µg) was electrophoresed on a 0.7% agarose gel, stained with ethidium bromide and visualised under WV light.

Please replace the paragraph starting at page 36, line 23 of the specification with the following amended paragraph:

pJEM11 plasmid DNA was purified from transformed *E. coli* DH10B cells. The plasmid DNA was quantitated based on its absorbance at 260 nm. Eleven micrograms of plasmid was digested to completion with 2.5 units of *Bam*HI at 37°C. for 2 h and was purified by agarose gel extraction. The resulting digested plasmid DNA was dephosphorylated using 2 units of alkaline phosphatase (Boehringer Mannheim, Germany) for 1 h at 37°C. to prevent recircularization ~~recircularisation~~ of the plasmid. The DNA was then purified by agarose gel extraction.

Please replace the paragraph starting at page 43, line 1 from bottom of the specification with the following amended paragraph:

To confirm the presence of the insert, PCR was carried out on five of the resulting colonies using the pMIP12 primers BlaF3 and R2 (see below), in the presence of Taq polymerase in 20 µl Eight microlitres from each PCR was electrophoresed on a 1% TAE agarose gel and ethidium bromide stained. The forward primer BlaF3 binds at approximately 150 base pairs within the *blaF** promoter and is designed to the coding strand. The reverse primer R2 binds 44 base pairs from the *Kpn* I site and is designed to the complementary strand of the transcriptional terminator. The expected size of the PCR product for the insert was approximately 900 base pairs.

BlaF3	5' TCGCGGGACTACGGTGCC (SEQ ID NO: 5)
R2	5' TCGAACTCGCCCGATCCC (SEQ ID NO: 6).

Please replace the paragraph starting at page 45, line 3 of the specification with the following amended paragraph:

In order to increase the likelihood of producing the protein of the invention in a form resembling that from the original host (*M. ptb*), it was expressed in the fast-growing species *M. smegmatis*. The ORF encoding the protein of the invention was cloned into the vector pMIP12 and used to transform *M. smegmatis*. To aid purification and detection of the recombinant protein, the gene was expressed with a C-terminal histidine.times.6 tag from the vector pMIP12. As shown in FIG. 3, the recombinant protein was detected from cell sonicates in both the soluble and insoluble fractions by Western blot analysis using a monoclonal anti-histidine x 6 POD conjugated antibody. The recombinant protein was clearly recognized ~~recognised~~ by the antibody (FIG. 3b) with no other signal obtained, confirming specificity for the histidine x 6 tag. The protein was further isolated from the soluble fraction by Ni^{+2}-affinity chromatography followed by elution with imidazole as described above. Recombinant protein could be seen in the 250 mM imidazole elution in SDS-PAGE gels with Coomassie Blue staining (see FIG. 3a). Western blot analysis showed the 250 mM imidazole elution contained most of the protein, with a slightly smaller amount present in the 40 nm elution and none was detected in the 1 M elution. There was a small amount of the protein still present in the flow-through, indicating that not all of the protein was bound to the column. This may be because the column was saturated with bound protein.

Please replace the paragraph starting at page 52, line 16 of the specification with the following amended paragraph:

Localization ~~Localisation~~ of the Protein of the Invention in M. ptb

Please replace the paragraph starting at page 53, line 6 of the specification with the following amended paragraph:

Localization ~~Localisation~~ of the protein in M. ptb

Please replace the paragraph starting at page 53, line 14 of the specification with the following amended paragraph:

The protein of the invention was prepared for immunization ~~imununisation~~ by transferring approximately 0.05 mg of Ni^{+2}-affinity enriched recombinant protein onto nitrocellulose membrane. The membrane was stained with Ponceau S and the protein band was excised, destained and air-dried. The blot was then fragmented inside a microfuge tube with the aid of a sterile scalpel blade. To this, approximately 300µl of PBS was added and the material was further fragmented until it could pass through an 18 gauge, 11/2" needle. Five hundred microlitres of Freund's incomplete adjuvant (F 5506, Sigma, USA) was added and the mixture was passed through the needle several times before being injected.

Please replace the paragraph starting at page 23, line 28 of the specification with the following amended paragraph:

To determine the cellular localization ~~localisation~~ of the native protein of the invention in *M. ptb* strain 316F, rabbit antibody was raised to recombinant protein. As shown in FIG. 10, the anti-serum recognized ~~recognised~~ recombinant protein on Western blots. Naive rabbit serum did not recognize ~~recognise~~ the protein. The serum was used to probe Western blots of equivalent amounts of *M. ptb* strain 316F culture filtrate. Results are shown in FIG. 11. A single band of apparent molecular weight 24.2 kDa was detected in the culture filtrate, which was slightly larger than the molecular weight of 22.3 kDa calculated for the mature native protein based on its amino acid composition. This band was also present in greater intensity in both the soluble and insoluble cell preparations. Several other weak bands of higher molecular weight were produced in the soluble and insoluble cell fractions with the naive and recombinant protein-immunized ~~immunised~~ rabbit sera Comparison to Ni^{+}-affinity chromatography prepared recombinant protein produced from *M. smegmatis*, previously shown to migrate with an apparent molecular weight of 23 kDa, confirmed native protein migrated slightly slower than recombinant protein.

Please replace the paragraph starting at page 55, line 10 of the specification with the following amended paragraph:

Southern blotting and hybridizations~~hybridisations~~

Approximately 1 μg of restriction endonuclease-digested genomic DNA fragments were electrophoresed in 0.7% agarose gels and transferred to nylon membranes (Biodyne B, Gelman, Pall Corporation, USA) by capillary transfer using standard procedures (Sambrook et al., 1989). The DNA was fixed to the membrane by exposure to UV light for 4 min using a Bio-Rad Gel Doc 2000 transilluminator (Bio-Rad, USA). Fixed membranes were prehybridized ~~prehybridised~~ in heat-sealed plastic bags with:DIG Easy Hyb buffer (Roche Molecular Biochemicals, Germany) at 42°C for 2 h with constant shaking in a Hot Shaker water bath (Bellco Biotechnology, N.J., USA). Hybridization ~~Hybridisation~~ was done in the same buffer at 2.5 ml/100 cm^2 with 25-50 ng/ml of denatured DIG-labeled probe DNA at 40°C for 18 h. The hybridized ~~hybridised~~ membranes were washed 2.times.5 min in 2 x SSC, containing 0.1% (w/v) SDS at room temperature, followed by 2 x 5 min washes in 0.7 x SSC containing 0.1% (w/v) SDS at 68°C. with constant shaking. Immunodetection of hybridized ~~hybridised~~ probe was achieved using the DIG system (Roche Molecular Biochemicals, Germany). Briefly, washed membranes were incubated in 1 ml/cm.sup.2 blocking solution (Roche Molecular Biochemicals, Germany) for 60 min at room temperature with shaking. The blocking solution volume was reduced to 20 ml/100 cm^2 and anti-DIG antibody conjugated to alkaline phosphatase (Roche Molecular Biochemicals, Germany) was added to a final dilution of 1:10,000 according to the manufacturer's recommendations. The blots were developed by chemiluminescence with CSPD or CPD-Star substrate (NEN, MA, USA). The developed blots were exposed to radiographic film (BioMax MR, Kodak, USA) for 5 min to 18 h, depending on signal intensity, in the presence of a single intensifying screen (Kodak Lanex Regular, Kodak, USA). Film was developed in an automated processor (Kodak RP X-OMAT Processor Model M6B).

Please replace the paragraph starting at page 56, line 8 of the specification with the following amended paragraph:

All probes were labeled by the incorporation of DIG-labeled dUTP (DIG-11-dUTP, Roche Molecular Biochernicals, Germany) during PCR. DIG-11-dUTP (1 573 152, Roche Molecular Biochemicak, Germany) was added to a final concentration of 20 μM in a reaction volume of 50 l and dTTP was adjusted to a final concentration of 80 μM. All other deoxynucleoside triphosphates were added to 100 μM. To estimate the purity and yield of DIG-labeled product, approximately 2 μl of the reaction was electrophoresed in agarose gels alongside a mass ladder for quantitation (10068-013 Low Mass DNA Ladder, Life Technologies Inc., USA). Due to the presence of DIG, the PCR products routinely appeared larger than unlabeled products. For quantitation of DIG-incorporation in probes, side-by-side filter spot tests, ranging from 0.01 pg to 10 pg, were carried out as per the manufacturer's recommendations. Labeled PCR products were stored at -20°C until used for hybridization ~~hybridisation~~.

Please replace the paragraph starting at page 57, line 21 of the specification with the following amended paragraph:

Southern blot analyses were performed with *Bam* HI-digested genomic DNAs from 13 *Mycobacterium spp.* The ORF encoding the protein of the invention was used as probe and was labeled by incorporation of DIG-dUTP during PCR using the primers 1 pp27-fBam and 1pp27-rKpn. FIG. 14 shows the probe hybridized ~~hybridised~~ strongly to a single band, approximately 2,200 base pairs in length from *M. ptb* 316F and a slightly larger band of 2,300 base pairs from *M. ptb* ATCC 53950. The probe also hybridized ~~hybridised~~ weakly to a 1,000 base pair band from *M. intracellularae*. Upon overnight exposure (data not shown), a weak band at approximately 10,000 base pairs was present in *M. marinum*, *M terrae*, *M pheli* and *M. kansasii*. Hybridization ~~Hybridisation~~ was not detected with *M. bovis*, *M. tuberculosis*, or *M. bovis* BCG.

Please replace the Abstract starting on page 72, line 2 of the specification with the following amended paragraph:

The present invention relates to an immunogenic polypeptide isolated from *Mycobacterium avium* ~~Mycobacterium avium~~ subspecies *paratuberculosis* ~~paratuberculosis~~ and variants of the polypeptide. The polypeptide and variants may be used in vaccines against Johne's disease and in methods for detection of the disease. Antibodies against the polypeptide or variants may be used in diagnostic tests for Johne's disease. Also included are polynucleotides encoding the polypeptide and variants, and methods for preparing these.